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United States Environmental Protection Agency
Office of Ground Water and Drinking Water
Technical Support Center

April 29, 2002

Dear Laboratory Manager:

Thank you for your interest in the U.S. EPA's Laboratory Quality Assurance Evaluation Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act (Lab QA Program). This is a voluntary program open to laboratories analyzing *Cryptosporidium* in water using EPA Method 1622 and EPA Method 1623. To increase the likelihood that laboratories analyzing water samples for *Cryptosporidium* generate reliable data, EPA has established the following process for evaluating laboratory performance and quality assurance practices:

Step 1. **Application.** Laboratories must first submit the Laboratory QA Program application. The application forms are enclosed with this letter, and the application requirements are described in detail below. EPA will evaluate laboratory applications to confirm the following: (1) the laboratory has the equipment required in EPA Method 1622 and/or EPA Method 1623 (April 2001), (2) laboratory personnel have the recommended experience to analyze samples, and (3) the laboratory has successfully completed the initial precision and recovery (IPR) and matrix spike/matrix spike duplicate (MS/MSD) tests specified in the method. Laboratories that do not meet these requirements will be requested to correct any deficiencies before proceeding to the next step in the evaluation process.

Step 2. **Performance evaluation.** After an application has been accepted, the laboratory will be sent a set of eight initial proficiency testing (IPT) samples consisting of a suspension of oocysts in a concentrated matrix. Laboratories will resuspend these spikes in reagent water to produce simulated source water samples, and analyze the samples using the version of Method 1622/1623 (April 2001) that the laboratory plans to use for routine *Cryptosporidium* analyses. If a laboratory wishes to be evaluated for more than one version of the method, the laboratory will receive a set of eight PT samples for each version.

Laboratory IPT data will be evaluated against the mean recovery and precision (as relative standard deviation) criteria for the IPT samples. Laboratories will receive two opportunities to pass the IPT test. If a laboratory fails two times, it will not be eligible for another set until after the laboratory staff has received additional training in performing the method.

Laboratories already participating in the EPA Protozoa Performance Evaluation (PE) Program, may use the initial round of samples from the PE program to meet the IPT sample requirement.

Step 3. **On-site evaluation.** After a laboratory passes the IPT, an on-site evaluation of the laboratory will be scheduled. The on-site evaluation will include two separate, but concurrent assessments: (1) assessment of the laboratory's sample processing and analysis procedures, including microscopic examination, and (2) evaluation of the laboratory's personnel qualifications, quality control program, equipment, and record keeping procedures.

Each laboratory will receive an audit report, which will document deficiencies, if any, that should be corrected by the laboratory. After a laboratory has corrected any deficiencies noted in the audit report, EPA will confirm that the laboratory meets the performance criteria of the Laboratory Quality Assurance Program for Analysis of *Cryptosporidium* under the Safe Drinking Water Act.

Laboratories that meet the program performance criteria will also receive a set of three ongoing proficiency testing (OPT) samples approximately every four months that must be analyzed in the same manner as the IPT samples. EPA will evaluate the precision and recovery data for OPT samples to determine if the laboratory continues to meet the performance criteria of the Laboratory QA Program.

Application Requirements

The first step in the laboratory evaluation process is submission of a laboratory application package. The following materials should be submitted for each laboratory application package:

1. Signed, completed application form (attached).

2. Completed self-audit checklist (attached). This checklist is similar to the checklist that will be used to audit your laboratory during the on-site evaluation.

3. Resumes detailing qualifications of your laboratory's proposed principal analyst/supervisor and each analyst and technician listed on the application form and documentation of the training, including the number of samples analyzed by each (the list for each analyst and technician should include at a minimum the number of samples specified below for personnel prerequisites).

   The recommended personnel prerequisites for the laboratory evaluation program are as follows:

   <u>Principal Analyst/Supervisor (one required per laboratory)</u>
   - BS/BA in microbiology or closely related field
   - A minimum of 1 year of continuous bench experience with *Cryptosporidium* and IFA microscopy
   - A minimum of 6 months experience using EPA Method 1622 and/or EPA Method 1623
   - A minimum of 100 samples analyzed using EPA Method 1622 and/or EPA Method 1623 (minimum 50 samples if the person was an approved analyst for *Cryptosporidium* under the Information Collection Rule(ICR))

   <u>Other Analysts (no minimum requirement per laboratory)</u>
   - Two years of college in microbiology or equivalent or closely related field
   - A minimum of 6 months of continuous bench experience with *Cryptosporidium* and IFA microscopy
   - A minimum of 3 months experience using EPA Method 1622 and/or EPA Method 1623
   - A minimum of 50 samples analyzed using EPA Method 1622 and/or EPA Method 1623 (minimum 25 samples if the person was an ICR-approved analyst)

   <u>Technician (no minimum requirement per laboratory)</u>
   - Three months experience with the specific parts of the procedure he/she will be performing
   - A minimum of 50 samples analyzed using EPA Method 1622 and/or EPA Method 1623 (minimum 25 samples if the person was an ICR-approved technician) for the specific analytical procedures they will be using.

4. Detailed laboratory standard operating procedures for each version of the method your laboratory plans on using for routine *Cryptosporidium* analyses. SOP's for the following should be included:

    - Performance of each method step including, sample spiking, filtration, elution, concentration, purification, slide preparation, sample staining and examination
    - Dividing pellets greater than 0.5mL
    - Preparation of reagents
    - Dishwashing
    - Staff training
    - Corrective action procedures for failing to meet OPR, method blank, staining controls, sample acceptance, and performance verification criteria
    - Sampling procedures to be followed by field or utility personnel
    - Procedures for data recording, checking manual calculations, and checking accuracy of all data transcriptions

5. EPA Method 1622 or EPA Method 1623 initial demonstration of capability (IDC) data which include initial precision and recovery (IPR) test results and matrix spike and matrix spike duplicate (MS/MSD) test results for *Cryptosporidium*. The IPR test consists of four reagent water samples spiked with between 100 - 500 oocysts and one method blank. The MS/MSD test consists of one unspiked and two spiked source water samples. These tests are described in Section 9 of EPA Method 1622 and EPA Method 1623 and the results should meet the criteria in the method (April 2001 version). The following data should be submitted:

    - Completed EPA Method 1622/1623 bench sheets and report forms for each of the eight samples (attached)
    - Initial demonstration of capability summary form (attached)
    - Spiking suspension preparation data. This should include completed spiking suspension sheets (attached) if spikes were prepared by your laboratory or flow-cytometer calibration forms if spikes were obtained from an external source.

    Laboratories wishing to be evaluated for more than one version of the method (different volumes, filters, elution and concentration procedures, and immunomagnetic separation kits) should submit a complete set of IPR and MS data for each version.

    If your laboratory currently participates in the EPA PT sample program and the required IDC data have already been submitted, the data do not need to be resubmitted. Please indicate this is the case on the initial demonstration of capability summary form.

6. Table of contents from your laboratory's quality assurance plan. The quality assurance plan should specifically address the requirements of protozoa analysis under the programs for which the laboratory intends to analyze samples.

7. An example of the data reporting form used to submit *Cryptosporidium* results to your clients.

8. A statistical summary of percent recoveries for all OPR and MS samples analyzed at your laboratory for the past six months.

Application materials should be submitted to the following address:

Jennifer Scheller
Cryptosporidium Laboratory QA Program Coordinator
DynCorp Biology Studies Group
6101 Stevenson Avenue
Alexandria, VA 22304

Send comments on the Agency's need for this information, the accuracy of the provided burden estimates, and any suggested methods for minimizing respondent burden, including through the use of automated collection techniques to the Director, Collection Strategies Division, U.S. Environmental Protection Agency (2822T), 1200 Pennsylvania Ave., NW, Washington, D.C. 20460. Include the OMB control number in any correspondence. Do not send the completed form to this address.

When your application package has been received and reviewed, you will be notified whether it is complete or has any deficiencies. After your application has been accepted, you will be notified of when you should expect your initial set of PT samples. If you have any questions about the laboratory application materials or evaluation process, please feel free to contact either me at 513-569-7944 or Jennifer Scheller at 703-461-2118.

Sincerely,

Mary Ann Feige
Cryptosporidium Laboratory Quality Assurance Evaluation Program Manager
26 West Martin Luther King Drive
Cincinnati, OH 45268

Attachments

Burden Statement: The public reporting and recordkeeping burden for this collection of information is estimated to average 18 hours per response or 72 hours per respondent annually. Burden means the total time, effort, or financial resources expended by persons to generate, maintain, retain, or disclose or provide information to or for a Federal agency. This includes the time needed to review instructions; develop, acquire, install, and utilize technology and systems for the purposes of collecting, validating, and verifying information, processing and maintaining information, and disclosing and providing information; adjust the existing ways to comply with any previously applicable instructions and requirements; train personnel to be able to respond to a collection of information; search data sources; complete and review the collection of information; and transmit or otherwise disclose the information. An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.